LIFE HISTORY OF *PRORODON GRISEUS*.

GEORGE W. TANNREUTHER,

ZOÖLOGICAL LABORATORY, UNIVERSITY OF MISSOURI.

INTRODUCTION.

Prorodon griseus, a Holotrichous ciliate, is somewhat plastic and variable in shape. When distended it is about two and one half times as long as wide, with its anterior end slightly wider (Figs. 1, 3). The oral aperture is sub-terminal. The anal pore is at the extreme posterior end. The pharynx is supported by twenty to thirty rod like structures. The contractile vacuole is postero-terminal. The delicate cuticular membrane is finely and closely striate longitudinally. No trichocysts are present. The cilia over the entire body are uniform in length, except at the anterior end where they are slightly longer. These longer cilia are quite active during the process of feeding. A layer of deeply stained bodies are found in the outer border of the ectoplasm. The locomotion of the expanded forms is rather smooth, rapid and straight forward, turning on their long axis. When contracted the motion is chiefly rotary.

The purpose of the following paper is to give a brief account of the more important features in the life history of *P. griseus*.

NATURAL HISTORY.

The material was collected from small rain pools, more especially from small hog wallows, which is one of their most favorable habitats for rapid growth and multiplication. When present in these small rain pools they are usually found in the surface film in rather large clumps, instead of being distributed throughout the water. When these small rain pools completely dry up abundance of encysted forms are found in the dry sediment on the sides and bottom. Collections were made at regular intervals (including encysted forms from the dry sediment), during the summer months of 1925. The material was placed in finger bowls with tap water added from time to time to counteract

the evaporation. Few free swimming forms were found when collected. But within twenty four hours great numbers would escape from the cysts. Conjugation occurred immediately at the beginning of the free swimming stage. The period of conjugation lasts from three to four or more hours. The ex-conjugants remained in the active swimming stage indefinitely, dependent upon external conditions.

Encystment is quite common, it may occur at any period in their life history and serves more for protection in carrying them over adverse conditions, due to extreme temperature or drought. The cysts in many cases are large, spacious and permit freedom of motion within (Fig. 2a). In the formation of the cysts the animal always contracts, assumes a spherical shape and rotates rapidly while secreting the cyst. The secretion is evenly distributed by the aid of the beating cilia. The animal usually forms its cyst in contact with some fine sediment, which acts more as a background. The cysts show considerable variation as to their flexibility and thickness (Fig. 2a–f). The cysts are either thin or temporary and thick or permanent. The animal may remain within the temporary cyst but a short time, then escape, and immediately begin the formation of a new cyst. Large numbers of cysts are often found in immediate contact and form a sort of network, as in Fig. 2. The animal just before escaping from the cyst becomes unusually active presses against one side of the cyst wall and breaks through. The permanent cysts differ from the temporary cysts, in that they are thicker, due to the secretion of new concentric layers within the first, until there is little space left for the animal within (Fig. 2f). After the permanent cyst is completed the animal becomes quiescent and remains in this condition indefinitely, at least until internal reorganization is complete.

In studying the formation of the cysts under the cover slip, it was found that the temporary cysts were often formed within thirty minutes, while the thick or permanent cysts required from three to five hours for their complete formation. In some of the permanent cyst under observation the animal remained quiescent for three days or more and then began a very rapid rotary motion within and soon escaped. The animal evidently must secrete

some substance which helps to dissolve the cyst wall, as it becomes thinner before their escape. No experiments were made to determine how long the encysted animals were able to survive under adverse conditions. A very small part of their life history, however, is passed in the free-living condition, even under favorable conditions as verified in the laboratory.

MATERIAL AND METHODS.

The material was fixed at various stages in the Life history of the animal. Bouin's fluid was found to give satisfactory results. The animals were removed from the cultures with the aid of a pipette avoiding excess water, and spurted into the tubes with the killing fluid. The centrifuge was quite helpful in concentrating the animals at one end of the tube. The specimens were stained in Delafield's hematoxylin or Tulodin blue. Either stain gave good results. Erythrosin was added to the ninety-five per cent. alcohol for cytoplasmic differentiation. The animals were then dehydrated, cleared in xylol and mounted in balsam. For sections the animals were imbedded in paraffine, sectioned and stained on the slide. The sections were quite helpful in checking the results as found in the whole mounts.

BINARY FISSION.

Reproduction by binary fission is quite common. It may occur during encystment (Fig. 2d), but it is found more abundant during the free active stages. Transverse division in the encysted stage is rather difficult to verify in the whole mounts, since the division of the cytoplasm as a rule can not be recognized except in sections. Division in the free-swimming stage is more distinct and the different stages can be followed step by step. The animal shortens somewhat on its long axis. The nuclear activities (Figs. 4–6) are similar to those of the encysted forms. The micro-nucleus as is common in ciliates divides first before division of the macro-nucleus. The macro-nucleus varies considerable in shape from a spherical to a somewhat elongated or curved condition. The elongated nucleus in binary fission often becomes constricted by a nuclear cleft without any further elongation and half going to either new daughter cell (Fig. 6).

Or again the macro-nucleus elongates more and more until the halves are connected by a mere thread and finally constricts when the separation of the cytoplasm in the formation of the daughter cells is complete (Fig. 5).

The new pharynx with its rod-like structures and the new contractile vacuole are formed before the nuclei divide. The entire process of binary fission requires about one and a half hours for completion. Binary fission occurs at any period in the life cycle of the animal. But the greater number of divisions were found to occur after conjugation during the active feeding stage. But if the conjugants encyst immediately after separation, due to adverse conditions, binary fission occurs within the cyst (Fig. 2*d*).

NUTRITION.

The direct observation of the feeding process in *Prorodon* is rather difficult, due to the rotary motion of the individuals when stationary or when swimming. The animal feeds chiefly upon bacteria and other small unicellular organisms. The origin, formation and fate of the food vacuoles is comparable to that of other ciliates where it is more easily followed. The animal at times is so gorged with food vacuoles, that a cytological study is impossible, since the body appears as a mass of indistinctly stained objects. Many of the individuals during feeding increase from two to three times in volume before binary fission occurs. This increase in size however, is due more to expansion than growth. The large mass of food vacuoles within the organism undergoes a constant movement in one direction for short intervals and then moves to and fro as a sort of churning. This constant circulation of the food vacuoles no doubt is greatly facilitated by the constant rotary motion of the organism. The food within the vacuoles undergoes a rapid digestion or lique-faction giving the vacuoles a clear content, which soon passes out into the cytoplasm. The food vacuoles as a rule are quite large, due to the great amount of fluid surrounding the food. Thus giving the appearance of a vesicle with a clear border and a dark center. The vacuoles finally collapse and the indigestible particles are voided at the posterior end through the anal pore.
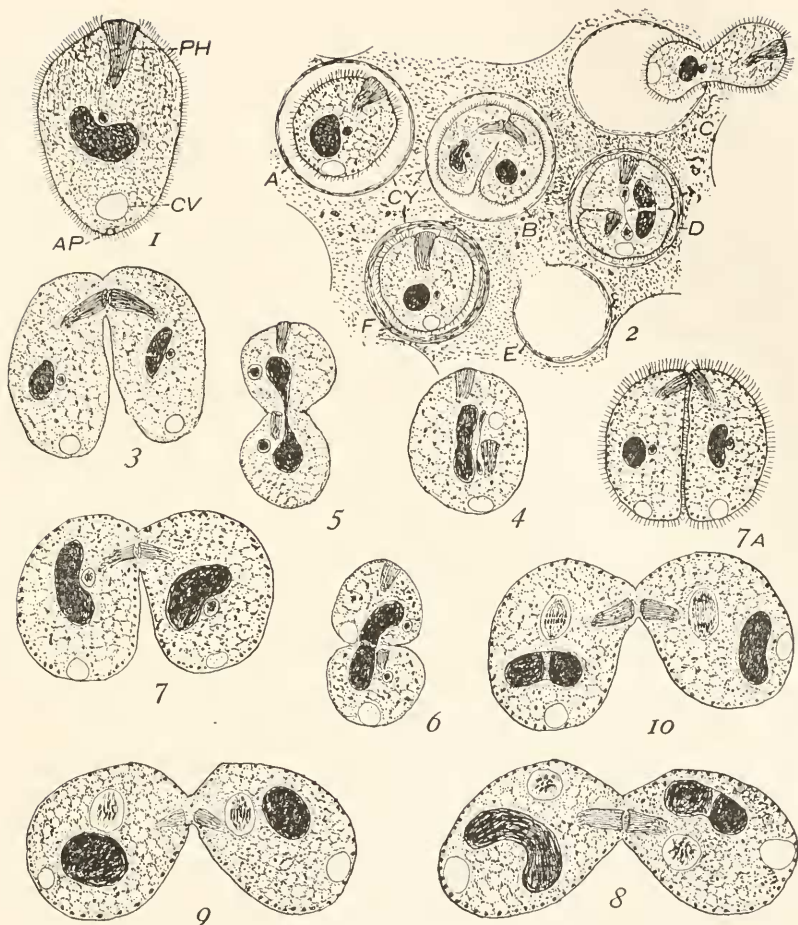
FIG. 1.   Whole mount drawing of Prorodon griseus to show structure. *ph.*, pharynx; *c. v.*, contractile vacuole; *a. p.*, anal pore.

FIG. 2.   Encysted forms; *a.*, temporary cyst; *b.*, conjugants within cyst; *c.*, escape from cyst; *d.*, binary fission within cyst; *e.*, cyst shell; *f.*, individual within permanent cyst.

FIG. 3.   Expanded conjugants.

FIGS. 4, 5 AND 6.   Different stages in the binary fission of free forms.

FIG. 7.   Conjugating individuals, early stage.

FIG. 7a.   Early conjugation before union of conjugants is effected.

FIGS. 8, 9 AND 10.   Micronuclei in different stages of the first maturation division.

CONJUGATION.

Conjugation occurs shortly after the animals escape from the cysts. In the early stages of conjugation the conjugants become spherical and press closely against each other, giving the semblance of binary fission. The animals rotate very rapidly on their long axes, slightly moving back and forth on the sides in contact (Fig. 7a). The anterior ends of the conjugants are slightly turned towards each other, so that the free end of either pharynx comes in contact. A small cytoplasmic bridge is then formed between the two individuals in close proximity and in front of either sub-terminal oral aperture. Thus the conjugation is terminal or end conjugation instead of lateral which is more common in the ciliates (Figs. 3, 8). The animals feed but little during conjugation. The conjugants often vary considerable in size. The period of conjugation lasts about four hours. When the union of the two animals is complete they elongate, with their anterior ends turned towards each other, and swim rapidly through the water, rotating on their long axes (Fig. 3). This method of activity may continue until conjugation is complete. But the greater number of conjugants observed during the process, became quiescent usually in contact with some sediment. They assumed a spherical form and rotated quite rapidly. While in this position a temporary cyst is often formed and the conjugation is completed within the cyst (Fig. 2b). If the cyst however, with the conjugants is disturbed they may escape before conjugation is complete and again move freely through the culture. A second temporary cyst is seldomly formed. This process of conjugation within a cyst is often taken for binary fission and that the two daughter cells produced within the cyst conjugated before escaping. This however, is not the case, since the first steps in conjugation of all individuals, as observed under the microscope, took place in the free swimming stage. The temporary encystment of the conjugants when it occurs is secondary and not essential except in sudden adverse conditions, since it does not occur in normal conditions if the forms are continually agitated during conjugation.

The relation of the conjugants in a straight line with their anterior ends united as the figures indicate, is due more to their

contraction during fixation (Figs. 8, 16). The nuclear activities during conjugation can best be followed in the encysted forms, or better perhaps in those that have just escaped from the cysts and become expanded, since they are freer from stained food vacuoles. The single micro-nucleus in the resting stage is always situated near the macro-nucleus, but never within it (Fig. 7).
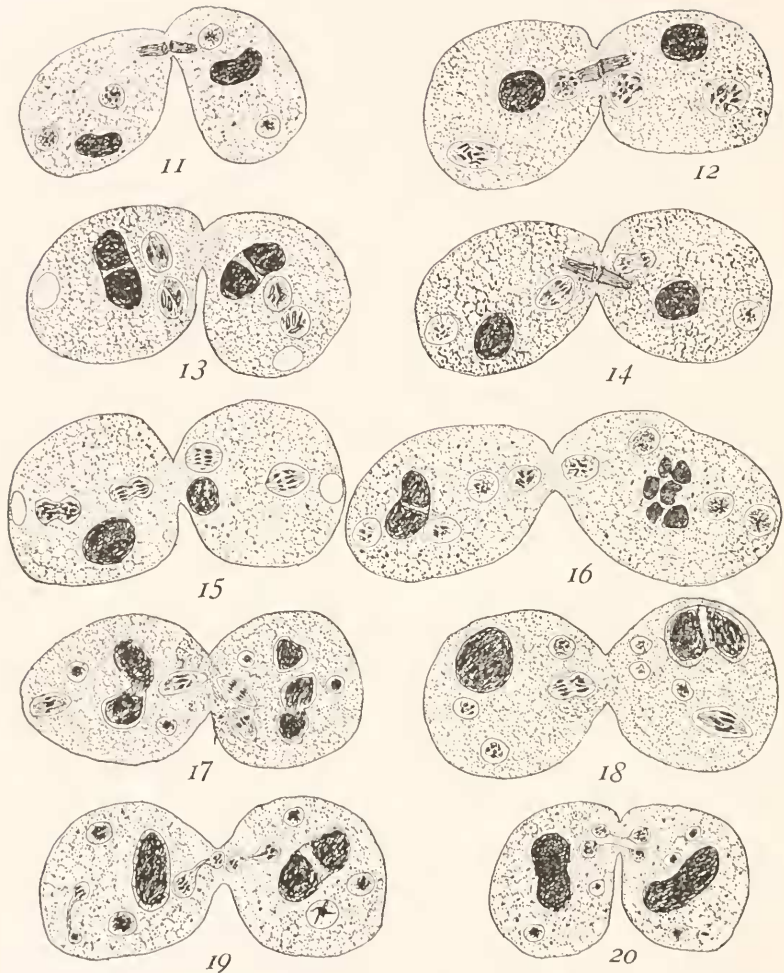
First Maturation Division.

The micro-nucleus at first stains as a homogeneous mass with a dark center (Figs. 1, 7). It soon enlarges, migrates towards the anterior end and becomes vesicular with a central chromatin mass of granules on a spireme, surrounded by a large clear area (Figs. 8, 16). The chromatin becomes concentrated into eight distinct staining bodies, which become elongated (Figs. 8, 9). In the first maturation spindle fibers extend towards either pole and the eight chromosomes divide transversely, giving rise to two equal masses or daughter micro-nuclei (Figs. 10–12). Immediately after division the two micro-nuclei pass into the resting stage (Fig. 11).

Second Maturation Division.

The second maturation division is similar to that of the first. Both micro-nuclei in either conjugant undergo division. The micro-nuclei with their darker staining center become vesicular (Figs. 11, 12). The chromatin at first in an irregular spireme becomes condensed into four double-staining bodies (Fig. 13), which elongate in the form of four double rods. The reduction of eight single rods to four indistinct double rods can be considered as the reduction stage. The four bivalent chromosomes become arranged on the equatorial plate parallel to the long axis of the spindle, and apparently divide transversely instead of longitudinally, as indicated in Figs. 14 and 15. At the close of the second division as in the first the chromatin passes into the resting or more vesicular condition before the next division (Fig. 16).

Third Division.

The four micro-nuclei resulting from the second division are about the same in size when first formed (Fig. 16). The four

FIGS. 11–15. Different stages in the second maturation division, showing the method of reduction of the chromosomes. Both micronuclei are dividing.

FIG. 16. Micronuclei produced by the second maturation division. The micronuclei are in the prophase stage of the third division. More than two micronuclei seldom divide in the same conjugant. The macronuclei are in the first stages of disintegration.

FIG. 17. Two of the micronuclei in either conjugant are dividing. The others are deteriorating. The macronuclei are dividing by nuclear clefts.

FIG. 18. A single micronucleus in either conjugant is dividing.

FIGS. 19 AND 20. Formation and exchange of pronuclei. The pronuclei vary in size, corresponding to moving and stationary nuclei.

micro-nuclei of either conjugant rarely undergo division. In most cases but two of the micro-nuclei divide, and occasionally but one divides. Those that do not divide show a very dark center at first, becoming fainter, taking up less and less stain and finally disappear within the cytoplasm by absorption (Figs. 17, 19). The activities in the origin, formation and division of the four chromosomes are quite distinct (Figs. 16–18). Fig. 17 shows two micro-nuclei in either conjugant in the process of division. The others are disintegrating. Fig. 18 shows but a single division in either conjugant. While in Fig. 19 there is one in one conjugant and two in the other which are dividing. The significance of this variation in the number of micro-nuclei in the third division is still an open question for explanation. The inactive micro-nuclei may persist indefinitely before their final absorption takes place. The micro-nuclei of the third division migrate toward the anterior end of either conjugant, where they complete their division and form the pronuclei (Figs. 19–21). The third division differs from the first and second maturation divisions in that there is an elongated connecting fiber between the two daughter nuclei. This connecting fiber is not entirely a part of the spindle, but is due more to the drawing out of the nuclear membrane before separation is complete (Figs. 19–21).

Pronuclei and their Fusion.

There is a slight difference of size in the pronuclei of either conjugant. The smaller can be considered as the moving pronucleus and the larger as the stationary pronucleus (Figs. 20–21). The interchange of the pronuclei is shown in Figs. 20 and 21. The fusion of the pronuclei occurs near the anterior ends of the conjugants (Figs. 22–23). In the formation of the conjugation nucleus (amphinucleus), the pronuclei at first are spherical and vesicular (Fig. 22). But later become slightly elongated. The sides of the nuclear membranes in contact may persist for awhile and prevent the direct fusion of the chromatin, giving the appearance of a double fusion nucleus (Fig. 23).

Cleavage and Formation of New Nuclei.

The number of divisions of the conjugation nucleus varies in different forms. It may divide once, twice, three or even four
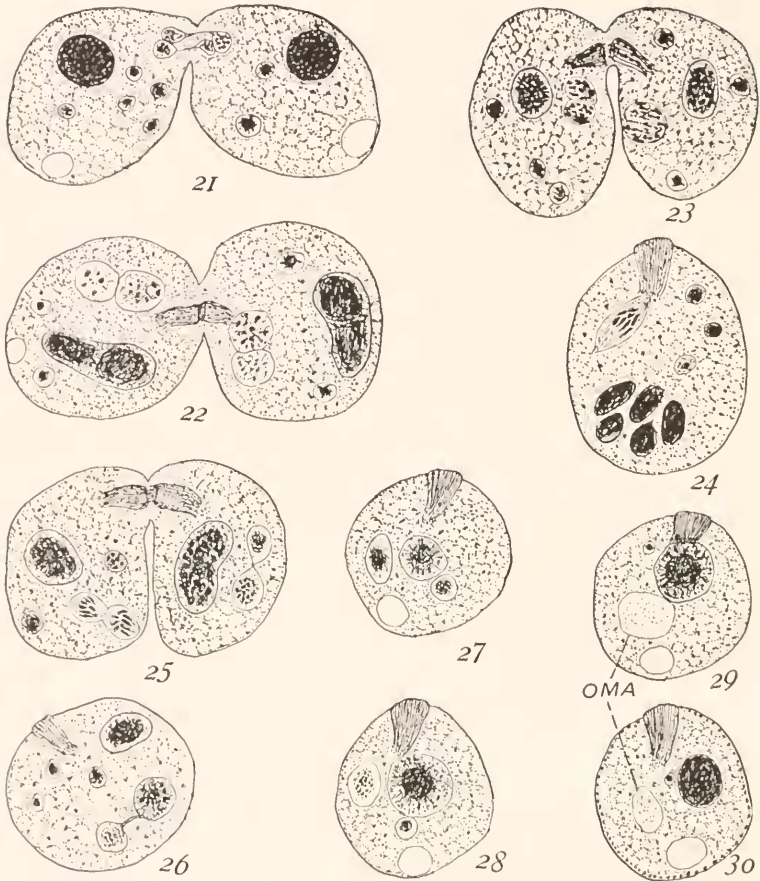
FIG. 21. Same as preceding figure with pronuclei slightly enlarged. Both macronuclei are spherical.

FIG. 22. Early stages in the fusion of the pronuclei.

FIG. 23. Later stages in the fusion of the pronuclei. The macronuclei are in their first stages of disintegration.

FIG. 24. Early cleavage stage of the fertilization nucleus. The macronucleus has divided into several parts.

FIG. 25. Later stages in the cleavage of the fertilization nucleus and the beginning of size differences in the daughter cleavage nuclei.

FIG. 26. The two cleavage nuclei remain connected by a delicate fiber in their early differentiation in the formation of the new micronucleus and the new macronucleus.

FIGS. 27–30. Different stages in the differentiation of the new micronucleus and the new macronucleus. Also the last stages in the disintegration and absorption of the old macronucleus, *o. ma.*, old macronucleus.

times, the latter case in *Bursaria truncatella*, before the differentiation of the new micro-nucleus and the new macro-nucleus occurs. In *Prorodon griseus* there is a single division. The conjugation nucleus immediately after its formation, enlarges somewhat and divides equally. The eight chromosomes are distinct and can be counted without difficulty. The two cleavage nuclei when first formed are quite similar in size and structure (Figs. 24, 25). The two daughter cleavage nuclei often remain connected by a delicate drawn out fiber, as shown in Fig. 26. One of the daughter cleavage nuclei decreases somewhat in size and produces the micro-nucleus. The other enlarges and becomes differentiated into the new macro-nucleus (Figs. 25, 26). The different stages in the formation of the new macro-nucleus are represented in Figs. 26 to 30. The chromatin network of the macro-nucleus at first is quite uniform or about the same texture throughout. The chromatin later, becomes quite distinct (concentrated) in the center, with a less dense spireme around its border (Figs. 27, 28). The dark center of chromatin enlarges until the nucleus is more or less homogeneous throughout (Figs. 29, 30). The new macro-nucleus at first is spherical, but later may assume different shapes as indicated above. The conjugants become separated during the formation of the new nuclei. According to Enriques ('08), in *Chilodon uncinatus* there is a single division of the conjugation nucleus and that the two daughter cleavage nuclei become differentiated into the new macro and the new micro-nucleus respectively.

The Old Macronucleus.

The macronucleus of the free swimming forms, as indicated above, is rather variable, ranging in shape from a circular form to an elongated condition, or at times it is somewhat curved. During early conjugation the nucleus when elongated shortens slightly and frequently divides into two or more parts (Figs. 16 and 17). But in most cases it does not divide at all (Figs. 20 and 21). The chromatin becomes more and more condensed and stains as a dark homogeneous body. The border of the dark staining nucleus fades out, leaving a dark center within a clear boundary (Figs. 23 and 25). Finally the dark center fragments

into many small bodies and disappears within the cytoplasm leaving a clear unstained outline (Figs. 28–30), which is later absorbed. The old macronucleus sometimes persists in the exconjugants after the formation of the new micronucleus and macronucleus are complete, before the beginning of its disintegration.

Occasionally when the macronucleus of one of the conjugants is situated at the anterior end in close proximity to the cytoplasmic bridge as in figure fifteen, it would be carried over to the opposite conjugant, due to the movement of the cytoplasm within. The two macronuclei within the same conjugant however, pass through the usual stages in their disintegration. This passing over of the macronucleus through the cytoplasmic bridge from one conjugant to the other is quite common in one of the species of *Chilodon*, where its passage can be followed in the living forms. In *Chilodon*, however, not only the macronucleus may pass in toto from one conjugant to the other, but the macronucleus of either conjugant divides, one of the resultant daughter nuclei acting as the moving nucleus and the other as the stationary nucleus, comparable to the micronuclear activities in normal conjugation.

General Remarks.

Conjugation and binary fission of the ciliate *P. griseus*, to my knowledge, has never been described. The general activities however, in reproduction for the most part agree with the results of investigators as found in other ciliates.

One of the chief points of interest in studying the different stages in the life history of the ciliate *P. griseus*, is the direct association of binary fission and conjugation with encystment. The entire process of cell division may occur within a given cyst. While the first steps in conjugation always occur (as far as my observations go), in the free swimming forms and encysts later when the union of the two conjugants is complete. Temporary encystment however, can not be considered as essential for binary fission and conjugation. Since both may occur in the free forms. Encystment here acts more as a protection in unusual or adverse conditions. In no instance observed did the daughter cells produced by binary fission within the cyst, undergo conjugation before their escape.

P. griseus differs from most Holotrichs, in the first stages of conjugation, in that each conjugant contracts, becomes sperical, and rotates vigorously. The sides in contact are somewhat flattened, with their sub-terminal oral apertures facing each other. They remain in this spherical shape until a point of cytoplasmic union is complete (Fig. 7a). Then they elongate (Fig. 3), and move freely through the culture until conjugation is complete. A greater number of the paired conjugants as a rule remain contracted, rotate on their long axes and secrete a temporary cyst in which they remain for the greater part or the entire period in the completion of conjugation (Fig. 2b).

In this connection it may be of interest to note that no conjugating individuals were found out of doors or under wild conditions. Although many of the pools were found in unfavorable conditions due to evaporation. In fact very few free-swimming forms were found at any one time when collections were made. Most of the individuals were encysted. But when placed under laboratory conditions, within a few hours the greater number of individuals escaped from the cysts, and became quite active. This was followed immediately by a regular epidemic of conjugation. Very few single individuals could be found in the cultures. Again within three to five hours very few conjugating individuals were present. The exconjugants during their free-swimming period fed quite actively and reproduced by binary fission. In about five hours encystment again occurred. Thus beginning a new cycle. A few active forms however, can be found in the cultures at all times.

It is maintained by Calkins and others that encystment (especially where cellular reorganization occurs), and conjugation are both conducive to renewed vitality. While by Mast and others it is held that neither encystment nor conjugation have any appreciable effect in producing new vigor.

In *P. griseus* however, we have conjugation immediately following encystment or even the conjugants may encyst during their nuclear exchange and reorganization. Here we might conclude that the exconjugants would receive unusual renewed vigor and increase the fission rate. This however, is not what we find in *P. griseus*. It is true that an epidemic of cell division

takes place in the exconjugants. But if we isolate an equal number of the exconjugants that encysted during conjugation, and an equal number of those that failed to conjugate immediately after encystment. We find that the rate of fission per given number is about the same in the two isolated lots. Hence it may be safe to conclude that simultaneous encystment and conjugation do not produce renewed vigor or at least increase the fission rate in *P. griseus*.
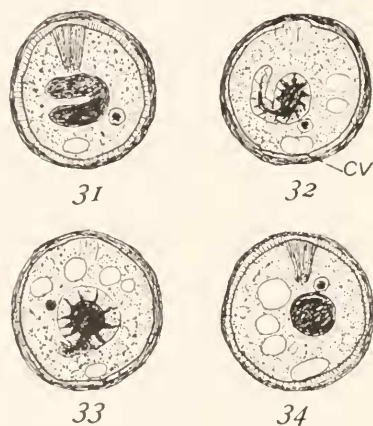

31   *32*

33   *34*

FIG. 31. Prorodon griseus in the early completion of the permanent cyst with all the organs in tact.

FIG. 32. Later stage of encystment with the disappearance of the cilia and the breaking up of the pharynx. The micronucleus and the contractile vacuole(?) persist. The macronucleus is in its early stage of metamorphosis, *c. v.*, contractile vacuole.

FIG. 33. The macronucleus in its later stage of metamorphosis. Several cytoplasmic vacuoles are present.

FIG. 34. Final stages of metamorphosis of macronucleus or cytoplasmic reorganization of structures during encystment. The missing organs have reformed. The new macronucleus is a direct metamorphosis of the old.

Encystment in the ciliates according to Calkins may serve a threefold purpose. That is for protection, reproduction and reorganization. In *P. griseus* there are two types of cysts which we have designated as thin or temporary and thick or permanent. The two types differ in the thickness and the compactness of their walls. The temporary cysts are quite flexible and yield to the moving ciliate within. The temporary cyst serves two purposes: first for protection against sudden adverse conditions such as

temperature, scarcity of food and evaporation. The ciliate if disturbed may remain in the temporary cyst less than an hour, and immediately after escape form a new cyst. The organism during temporary encystment does not undergo any internal changes. It maintains its spherical shape and continues to rotate. The second purpose is for reproduction by transverse division. Likewise conjugation, except the early fusion of the conjugants, may occur within the cyst. The third purpose is for the reorganization of the cell. The cyst in this case is of the permanent type, its walls being thick and dense. The individual becomes quiescent, and loses its cilia and pharynx during internal reorganization (Figs. 31–33). In this latter case the organism can not be recovered from the cyst within a period of at least four or more days or until its reorganization is complete.

The micro-nucleus and contractile vacuole persist throughout the reorganization without undergoing any appreciable change. The macro-nucleus however passes through a distinct series of changes as indicated in Figs. 31 to 34. Fig. 31 represents the condition at the beginning of encystment when all of the organs are present. The macro-nucleus is horse-shoe shaped, but in many individuals it is spherical. In the first steps of macro-nuclear metamorphosis the chromatin becomes ragged (Fig. 32), with a clear border beneath the nuclear membrane. The central region of the chromatin remains dark. Fig. 33 shows a third stage in which the chromatin has taken a more spherical shape with the ragged edges fraying out. Finally in Fig. 34 the macro-nuclear change is complete, the more central portion of the chromatin persisting as the new nucleus. The organs which were lost have reformed.

The macro-nuclear changes in *P. griseus* are similar to the conditions found by Stolte, in *Blepharisma undulans* and by Brand, in *Vorticella microstoma* and in *Stylonychia mytilus*. The macro-nuclear activities during encystment are considered by them as a cytoplasmic reorganization process since no new nuclei are formed. According to Stolte cytoplasmic depression, occasioned by adverse external conditions leads to encystment, and nuclear depression leads to conjugation. In *Didinium nastutum* however, as reported by Calkins and in *Stylonychia pustulata* as

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reported by Fermor, the macro-nucleus disintegrates and a new micro-nucleus and a new macro-nucleus are formed from the old micro-nucleus, thus undergoing a complete nuclear reorganization comparable to conjugation.

General Summary.

1. *Prorodon griseus* is a Holotrichous ciliate. It is recognized by its sub-terminal oral aperature, its rod like structure enclosing the pharynx, and its terminal contractile vacuole.

2. *P. griseus* has a single micronucleus and a single macronucleus.

3. Binary fission and conjugation occur either while encysted or in the free forms.

4. Encystment serves for protection, reproduction and reorganization.

5. Cysts are of two kinds: (*a*) temporary for protection during short intervals. (*b*) permanent for reorganization of cell and protection for long intervals, from one cycle to the next.

6. Conjugation is terminal, fusion occurring at their anterior ends. Conjugation occurs immediately after the animals escape from the cysts.

7. The animals are found encysted during the greater part of their life history. The greater number of free forms are found immediately after conjugation, during the active feeding period.

8. Encystment and conjugation in *P. griseus* have little or no effect in the production of new vigor and in the change of fission rate.

9. The old macronucleus persists during early conjugation, then disintegrates and disappears by absorption within the cytoplasm.

10. The new micronucleus and macronucleus are formed from the daughter nuclei, produced by a single division of the conjugation nucleus.

11. The reduction of chromosomes occurs in the second maturation division by a pairing of the eight chromosome in the formation of the four bivalent structures.

12. In the third division the pronuclei at first are connected by a drawn out fiber.

13. The pronuclei elongate slightly during their process of fusion.

14. In cellular reorganization within the permanent cyst, the cilia and pharynx disappear. The micronucleus and contractile vacuole (?) persist. The macronucleus passes through a distinct metamorphosis.

15. Reorganization during permanent encystment in *P. griseus* is of the cytoplasmic or passive type. Nuclear reorganization or formation of a new micronucleus and a new macronucleus from the old micronucleus was not found to occur.

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